

ANTIGENIC COMPOSITION COMPRISING A POLYCATIONIC PEPTIDE AND INOSINE AND CYTOSINE

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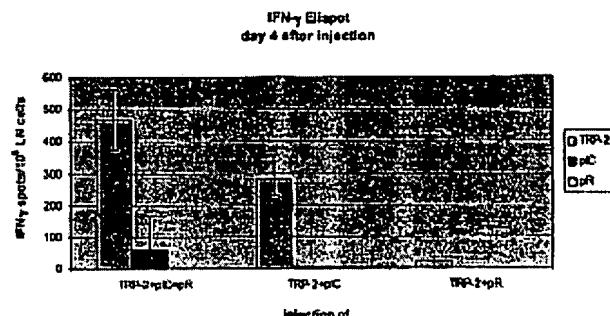
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Abstract of WO0193903

The invention relates to a composition comprising: a T cell epitope or a mixture of T cell epitopes, a polycationic peptide and a nucleic acid based on inosine and cytosine and its use as a vaccine.



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ANTIGENIC COMPOSITION COMPRISING A POLYCATIONIC PEPTIDE AND INOSINE AND CYTOSINE

A vaccine can contain a whole variety of different antigens.

Examples of antigens are whole-killed organisms such as inactivated viruses or bacteria, fungi, protozoa or even cancer cells.

Antigens may also consist of subfractions of these organisms/tissues, of proteins, or, in their most simple form, of peptides.

Antigens can also be recognized by the immune system in form of glycosylated proteins or peptides and may also be or contain polysaccharides or lipids. Short peptides can be used since for example cytotoxic T cells (CTL) recognize antigens in form of short usually 8-11 amino acids long peptides in conjunction with major histocompatibility complex (MHC) (Rammensee et al., 1995).

In order to obtain sustained, antigen-specific immune responses, adjuvants need to trigger immune cascades that involve all cells of the immune system necessary. Primarily, adjuvants are acting, but are not restricted in their mode of action, on so-called antigen presenting cells (APC), of which dendritic cells (DCs) are the most potent. These cells usually first encounter the antigen (s) followed by presentation of processed or unmodified antigen to immune effector cells. Intermediate cell types may also be involved. Only effector cells with the appropriate specificity are activated in a productive immune response.

The adjuvants may also locally retain antigens and co-injected other factors. In addition, the adjuvants may act as a chemoattractant for other immune cells or may act locally and/or systemically as a stimulating agent for the immune system.

Cells of the innate immune system recognize patterns expressed on their respective targets. Examples are lipopolysaccharides (LPS) in the case of Gram-negative bacteria, mycobacterial glycolipids, lipoteichoic acids of Gram-positive bacteria, mannans of yeast and double stranded RNAs of viruses (Hoffmann et al., 1999). In addition, they may recognize patterns such as altered glycosylations of proteins on tumor cells.

Polycationic polymers, for example the polycationic amino acid polymers poly-L-arginine and poly-L-lysine, have been shown to allow very efficient charging of APC with antigens *in vitro* and *in vivo* (Buschle et al., 1998, Buschle et al., 1997, Schmidt et al., 1997). This is thought to be the key event for triggering immune cascades, eventually leading to the induction of antigen specific immune effector cells that are able to destroy or neutralize targets. It has been shown previously that a number of polycationic compounds exert effects on immune cells (Buschle et al., 1998, Buschle et al., 1997).

Co-injection of a mixture of poly-L-arginine or poly-L-lysine together with an appropriate antigen as a vaccine protects animals from tumor growth in several animal models (Buschle et al., 1998, Schmidt et al., 1997). This chemically defined vaccine is able to induce a high number of antigen-specific T cells. In order to induce antigen-specific T cells, peptides need to be taken up by APC. Such peptide-loaded APC will induce an immune cascade, eventually leading to the induction of antigen-specific immune effector cells like T cells.

Polyinosinic-polycytidylic acid (poly I: C) is known as a potent interferon type I inducer (Manetti et al., 1995). Because of its protective effects in a number of animal species against a broad spectrum of both RNA and DNA viruses (e. g., herpes simplex virus, rabies virus, Japanese B encephalitis virus, vaccinia

virus, encephalomyocarditis virus), poly I: C is often used in models of viral infections. Changes that occur in response to poly

I: C are thought to be representative of changes that occur in response to a variety of different viruses. Poly I: C is known to stimulate macrophages to produce cytokines such as IL-1 α and IL-12 (Manetti et al., 1995), it is a potent NK cell stimulator (Cavanaugh et al., 1996) and, in general, this compound is known to promote Th1-specific immune responses. Because of these abilities, poly I: C has been widely applied as an immunomodulator in several clinical trials showing little or no toxicity (Guggenheim et al., 1977, Simnaler et al., 1977). However, there was no patient benefit. It is unclear whether poly I: C on its own has adjuvant activity. Recent findings show that poly I: C also induces stable maturation of in vitro-cultured DCs and that such DCs are potent T cell stimulators in vitro (Cella et al., 1999; Verdijk et al., 1999).

It is an object of the present invention to provide an effective vaccine system to allow an effective delivery of a specific antigen to the immune system of man or animal to achieve an efficient immunization against such an antigen.

This object is solved by a pharmaceutical composition comprising a T cell epitope (i. e. an antigen recognized by T cells) or mixtures of T cell epitopes, a polycationic peptide and a nucleic acid based on inosine and cytosine.

It has surprisingly turned out that, although polycationic peptides and nucleic acids based on inosine and cytosine have been previously known as being efficient adjuvant substances, the combination of both substances with a T cell epitope shows synergistic effects in immunostimulation which by far exceeds their additive contributions.

Moreover, whereas in previous uses of polycationic peptides and/or nucleic acids based on inosine and cytosine in vaccines only large antigens or whole cell vaccines have been used (where no synergistic effect of the two components may be seen), it could be shown by the present invention that on a T cell level antigens may be provided in a vaccine which gives an efficient T cell response. Immunisation with large antigens or even whole cells, results in the generation of antibodies. According to the present invention, such antibody generation is not an object.

Therefore, the compositions according to the present invention preferably contain antigens lacking B-cell epitopes and containing only antigens having one or more specific T cell epitope (s).

Indeed, it could be shown that also weakly immunogenic T cell epitopes which normally give no T cell response ("normally" = either alone, with adjuvants according to the prior art or with only one immunostimulatory substance according to the present invention), may be formulated into very efficient T cell vaccines by combining it with a polycationic polypeptide and a nucleic acid based on inosine or cytosine. Therefore, vaccines according to the present invention may also comprise T cell epitopes which do not result in a sufficient T cell response under "normal" conditions (i. e. in vaccine compositions according to the prior art as defined above). The term "antigen" hereinafter relates to "T cell epitope(s)".

Especially antigens may be successfully used in the present invention, which do not result in a sufficient immune response when applied with alum, the standard adjuvant (or other prior art adjuvants are disclosed e. g. in Singh et al. (Nat. Biotechnol. 17 (1999), 1075-1081)) or with polycationic polypeptides or nucleic acids based on inosine and cytosine alone. A sufficient immune response may be regarded e. g. resulting in more than 50, preferably more than 200, especially more than 400 IFN-spots/10⁶ unseparated cells in an Elispot-assay.

Preferably, the nucleic acid based on inosine and cytosine is selected from poly I : poly C, poly IC, poly dC: poly dI and poly dIC. Of course, any other combinations of complementary double-stranded IC

sequences are also preferred as well as chemically modified nucleic acids, e. g. thiolated poly. 1C as described in US 6 008 334; US 3,679,654 and US 3,725,545.

Polycationic peptides to be used in the present invention are e. g. described in the W097/30721. Preferred polycationic peptides are poly-lysines, poly-arginines and poly-peptides containing more than 50% of basic amino acids, especially arginine or lysine residues, in a range of more than 5, especially more than 8 amino acid residues or mixtures thereof. These polycationic peptides may be produced chemically or recombinantly or may be derived from natural sources. Preferred polycationic peptides derived from natural sources include HIV-rev or HI-tat derived cationic peptides, antennapedia peptides, cationic antimicrobial peptides, defensins, chitosan (or other derivates of chitin) and other peptides derived from these peptides or proteins by a biochemical recombinant production. Preferably, the polycationic peptides contain between 10 and 1000 residues, especially between 50 and 500 residues. Preferably, these peptides contain more than 70%, especially more than 85% basic amino acid residues, such as arginine, lysine, ornithine etc., and also synthetic organic polycations (polypeptide-like substances), like polyethyleneimine, histones, protamine, as disclosed in the W097/30721. Of course, also mixtures of different polycationic peptides or polypeptides may be used.

The antigens to be used in the present composition are not critical. Preferably, peptides derived from a viral or a bacterial pathogen or from fungi or parasites are used as such antigens (including derivatized antigens or glycosylated or lipidated antigens or polysaccharides or lipids). Preferred (human, animal and plant) pathogens are selected from HIV, HBV, HCV, Influenza virus, Rotavirus, *Staphylococcus aureus*, *Chlamydia pneumoniae*,

Mycobacterium tuberculosis, *Streptococcus pneumoniae*, *Bacillus anthracis*, *Vibrio cholerae*, *Plasmodium* sp. (Pl. *falciparum*, Pl. *vivax*, etc.), *Aspergillus* sp. or *Candida albicans*. The derivation process may include the purification of a specific protein from the pathogen, the inactivation of the pathogen as well as the proteolytic or chemical derivatization or stabilization of such a protein. Alternatively, also parts of the pathogen itself may be used as an antigen. In the same way also tumor antigens (cancer vaccines) or autoimmune antigens may be used in the pharmaceutical composition according to the present invention. With such compositions a tumor vaccination or a treatment for autoimmune diseases may be performed.

The antigens are preferably peptide or protein, carbohydrate or lipid antigens or mixtures thereof. Antigens from parasites or plant pathogens are also preferred.

Preferably, the antigen is a peptide consisting of 6 to 20, preferably, 7 to 15, especially 8 to 11, amino acid residues. Antigens of this length have been proven to be especially suitable for T cell activation.

According to another aspect the present invention relates also to the use of a composition according to the present invention for the preparation of a vaccine.

The relative amounts of the ingredients of the present composition are highly dependent on the necessities of the individual composition, e. g. the polycationic peptide to be used.

Preferably between 1 ug and 1 g of antigen, polycationic peptide and nucleic acid based on inosin and cytosin are applied. In case of poly-L-arginine and poly-L-lysine, preferred amounts of antigen/polypeptide/nucleic acid based on inosine and cytosine lie in the range of 1-10000jig antigen per vaccination and 0.1 to 1000 ug polypeptid. The amount of poly IC may preferably range from 1 to 5000pg/kg body weight(10 ug-500 mg).

The composition according to the present invention may further contain auxiliary substances, such as buffers, salts, stabilizers, antioxidants, etc., or other effective substances, such as antiinflammatory or antinociceptive drugs.

The present compositions may be applied to a patient, e. g. a vaccination candidate, in efficient amounts, e. g. by weekly, biweekly or monthly intervals. Patients to be treated with the present composition may also be vaccinated repeatedly or only once.

A preferred use of the present invention is the active immunization, especially of humans or animals without protection against the specific antigen.

The route of application for the present composition is not critical, e. g. subcutaneous, intramuscular, intradermal or trans dermal injection is suitable as well as oral uptake. It is also possible to apply the present composition separately, e. g. by injecting the nucleic acid based on inosine and cytosine separately from the antigen/polycationic peptide composition. The present invention is therefore also directed to a kit comprising a composition containing the antigen and the polycationic peptide as one component and a composition containing the nucleic acid based on inosine and cytosine as a second component. The components may be applied at the same time or site, however, an application at different sites or at a different time or for a different time period is also possible. It is also possible to vary the systemic or local applications of the composition or the components, respectively.

The present invention further relates to the use of the compositions according to the present application for the preparation of a vaccine inducing a systemic immune response.

Details of the present invention are described by the following examples and the figures, but the invention is of course not limited thereto.

Fig. 1 shows the immune response against ovalbumin-derived peptide of a combined injection of polyinosinic-polycytidylic acid(pIC) and poly-L-arginine (pR); Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with Ova-derived peptide, pIC or pR.

Number of IFN- γ -producing cells was determined 24 hours later using an ELISPOT assay. Results are expressed as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 2 shows the immune response against ovalbumin-derived peptide of a combined injection of pIC and pR; Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with Ova-derived peptide, pIC or pR. Number of IFN- γ -producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 3 shows the induction of ovalbumin-peptide specific T cells after combined injection of pIC and pR; Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with Ova-derived peptide, pIC or pR. Number of IFN- γ -producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 4 shows the immune response against a TRP-2-derived peptide after combined injection of pIC and pR; Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with TRP-2-derived peptide, pIC or pR. Number of IFN- γ -producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 5 shows the induction of T cells specific for a P1A (mastocytoma-derived) peptide after combined

application of pIC and pR; Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with

P1A peptide, pIC or pR. Number of IFN-Y-producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 6 shows the induction of T cells specific for Ova-derived peptide after combined application of oligo-dIC26 and pR;

Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with Ova-derived peptide, oligo-dIC26 or pR. Number of IFN-y-producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 7 shows that the combined injection of OVA-derived peptide with pR and pIC induces systemic antigen-specific T cell response; mice were injected subcutaneously either into hind footpads (A) or into the flank (B) with mixtures as indicated. On day 7 after injection, IFN-y-ELISPOT was carried out with peripheral blood leucocytes (PBLs), which were re-stimulated with OVA257264 peptide. The number of IFN-y-producing cells was determined 24 hours later. Results are shown as the number of spots/lx10⁶ PBLs SD of duplicates.

Fig. 8 shows that the combined application of OVA-derived peptide with pIC and various polycationic compounds strongly enhances the induction of peptide-specific T cells. Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with the relevant peptide (OVA257-264 peptide), irrelevant peptide (TRP-218i-18s peptide), pIC, or the respective polycationic compound. Number of IFN-y-producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 9 shows that the combined application of ovalbumin (OVA) with pIC and pR strongly enhances the induction of OVA-specific T cells. Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with

MHC class I- and II-restricted OVA-peptides or OVA. The number of IFN-y-producing cells was determined 24 hours later using an EL

ISPOT assay. The results are shown as the number of spots/lx10⁶ LN cells SD of triplicates.

Fig. 10 shows that Poly-L-arginine (pR) does not affect polyinosinic-polycytidylic acid (pIC)-induced in vitro maturation of human DCs. To determine phenotypic maturation, either pIC, pR, both pIC and pR or, for control purposes, LPS and medium alone was added to day 6-cultured human DCs. Extensive phenotypic analysis of surface antigens was performed after 48 hours of stimulation.

Fig. 11 shows that the combined application of OVA-derived peptide with oligo-dIC26mer and pR results in the systemic, antigen-specific T cell response. Mice were injected subcutaneously into the footpad with mixtures as indicated in the Figure legend.

At selected time points after injection, spleen cells (SCs) or peripheral blood leukocytes (PBLs) were isolated and re-stimulated with OVA-derived peptide. The number of IFN-y-producing cells was determined 24 hours later using an ELISPOT assay. The results are shown as the number of spots/lx10⁶ cells SD of duplicates.

Fig. 12 shows that the combined application of ovalbumin (OVA) with oligo-dIC26-mer and pR strongly enhances the induction of OVA-specific T cells. A) Mice were injected subcutaneously into the footpad with mixtures as indicated. Four days later, draining lymph nodes (LN) were taken and lymph node cells were re-stimulated either with MHC class I- and II-restricted OVA-derived epitopes or OVA. The number of IFN- γ -producing cells was determined 24 hours later using an ELISPOT assay. The results are shown as the number of spots/ 1×10^6 LN cells SD of triplicates.

B) At selected time points after injection, peripheral blood leukocytes (PBLs) were isolated and re-stimulated with OVA257-264-peptide. Number of IFN- γ -producing cells was determined 24 hours later using an ELISPOT assay. Results are shown as the number of spots/ 1×10^6 PBLs SD of duplicates.

Fig. 13 shows that the combined application of ovalbumin (OVA) with oligo-dIC26-mer and pR enhances production of OVA-specific IgG antibodies. Mice were injected subcutaneously into the footpad with mixtures as indicated. At day 24 and 115 after injection, sera were collected and screened by ELISA for OVA-specific IgG2a (A) and IgG1 (B) antibodies. The results are shown as the antibody titer.

EXAMPLES

Example 1

The combined injection of polyinosinic-polycytidylic acid (pIC) and poly-L-arginine (pR) synergistically enhances the immune response against ovalbumin-derived peptide.

Mice C57B1/6 (Harlan/Olac)

Peptide VA257264-Peptide (SIINFEKL), an

MHC class I (H-2K b)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), was synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spectroscopy for purity.

Dose: 300 μ g/mouse

Poly-L-Arginine (pR) Poly-L-Arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100 μ g/mouse

Polyinosinic-polycytidylic acid polyinosinic-polycytidylic acid (pIC) (Sigma Chemicals, P-0913, Lot 125H4024) with the molecular weight between 220,000 to 460,000

Dose: 100 μ g/mouse

Experimental groups (4 mice per group) 1. OVA257-264-peptide + pIC + pR 2. OVA257-264-peptide + pIC 3. OVA 257-264-peptide + pR

On day 0 mice were injected into each hind footpad with a total volume of 100 μ l (50 μ l per footpad) containing the above mentioned compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Single cell suspensions were prepared by mincing lymph nodes through a 70 μ m cell strainer. Thereafter, cells were washed twice with DMEM medium (Life Technologies) containing 5% fetal calf serum (FCS, SIGMA Chemicals). Cells were adjusted to 10 7 cells/ml in DMEM/5% FCS.

IFN- γ ELISPOT assays were carried out in triplicates as described

(Miyahira et al., 1995). This method is a widely used procedure for the quantification of antigen-specific T cells. Lymph node cells were re-stimulated in vitro either with Ova-peptide, polyinosinic-polycytidylic acid (pIC), poly-L-arginine (pR), Concanavalin A (ConA) or medium alone (background). Each spot represents a single IFN- γ -producing T cell. Spots were counted using a Biosys reader (Biosys, Germany). Number of background spots was subtracted from all samples. The results are expressed as the number of spots/ 1×10^6 unseparated cells SD of triplicates. After the stimulation with ConA we could detect many

spots (data not shown) indicating a good condition of the used lymphocytes. As shown in Figure 1, by injecting Ova-derived peptide with a combination of pIC and pR, we could induce almost 800 peptide-specific T cells among one million lymph node cells. In contrast, injections of peptide with either of the substances alone failed to induce peptide-specific T cells (Figure 1).

Example 2

The combined injection of pIC and pR enhances the immune response against ovalbumin-derived peptide in a concentration(pIC)-dependent manner.

MiceC57B1/6 (Harlan/Olac)

PeptideOVA2 57-264-Peptide (SIINFEKL), an MHC class I (H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), was synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spectroscopy for purity.

Dose: 300ug/mouse

Poly-L-Arginine (pR) Poly-L-Arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100pg/mouse

Polyinosinic-polycytidylic polyinosinic-polycytidylic acid acid (pIC) (Sigma Chemicals, P-0913, Lot 125H4024) with the molecular weight ranging from 220,000 to 460,000

Dose: 100,50,25ug/mouse

Experimental groups (4 mice per group) 1. OVA257-264-peptide + pIC 100jig + pR 2.OVA-peptide + pIC 50ig + pR3. OVA 257-264-peptide +pIC 25ig + pR 4. OVA257-264-peptide + pIC 100 g 5.

OVA257-264-peptide + pR

On day0 mice were injected into each hind footpad with a total volume of 100 ul (50pi per footpad) containing the above mentioned compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph node cell suspensions were prepared and IFN- γ ELISPOTs were performed as described in example 1. Results are expressed as the number of spots/1x10⁶ cells SD of triplicates. Even very low dose of pIC (25pg/mouse) injected in a combination with pR plus peptide leads to the induction of antigen-specific T cells (Figure 2).

Example 3

The combined injection of pIC and pR enhances the induction of ovalbumin-peptide-specific T cells in a peptide concentration-dependent manner.

MiceC57B1/6 (Harlan/Olac)

Peptide VA257264-Peptide (SIINFEKL), an MHC class I(H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), was synthesized using standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity.

Dose: 300,100,50ug/mouse

Poly-L-Arginine (pR) Poly-L-Arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100ug/mouse

Polyinosinic-polycytidylic polyinosinic-polycytidylic acid acid (pIC) (Sigma Chemicals, P-0913, Lot 125H4024) with the molecular weight ranging from 220,000 to 460,000

Dose: 100qg/mouse

Experimental groups (4 mice per group) 1. VA257264-peptide 300 g + pIC + pR 2.OVA-peptide 100 g + pIC + pR3. OVA-peptide 50 ug + pIC + pR4. OVA 257-264-peptide 300 ug + pIC 5. OVA257-264-peptide 300 ug + pR

On day 0 mice were injected into each hind footpad with a total volume of 100 ul (50 ul per footpad) containing the above mentioned compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph node cell suspensions were prepared and IFN- γ ELISPOTs were performed as described in example 1. Results are expressed as the number of spots/1X10⁶ cells SD of triplicates. As shown in Figure 3, comparably strong immune response can be induced even when lower peptide dose (100 ug instead of 300ug/mouse) is used for the vaccination.

Example 4

The combined injection of pIC and pR synergistically enhances the immune response against a TRP-2 (mouse tyrosinase-related protein-2)-derived peptide.

Mice C57B1/6 (Harlan/Olac)

Peptide TRP-2-Peptide (VYDFFVWL), an MHC class I(H-2Kb)-restricted epitope of mouse tyrosinase-related protein-2 (Bloom et al., 1997) was synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity.

Dose: 100ug/mouse

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100 ug/mouse

Polyinosinic-polycytidylic polyinosinic-polycytidylic acid acid (pIC) (Sigma Chemicals, P-0913, Lot 125H4024) with the molecular weight ranging from 220,000 to 460,000

Dose: 100 ug/mouse

Experimental groups (4 mice per group) 1. TRP-2 + pIC + pR 2. TRP-2 + pIC 3. TRP-2 + pR

On day 0 mice were injected into each hind footpad with a total volume of 100 pi (50pi per footpad) containing the above mentioned compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Preparation of lymph nodes and IFN-ELISPOTs were carried out as described in example 1. Results are expressed as the number of spots/1X10⁶ cells + SD of triplicates. Our results show that pIC and pR also synergistically act in inducing TRP-2 peptide-specific T cells (Figure 4).

Example 5

The combined application of pIC and pR strongly enhances the induction of T cells specific for a mastocytoma-derived peptide.

Mice DBA/2 (Harlan/Olac)

Peptide Mouse mastocytoma P815-derived peptide P1A (LPYLGWLVF), restricted to MHC class I(H2-Ld) (Lethe et al., 1992), synthesized by standard solid phase F-moc synthesis, HPLC purified and analysed by mass spectroscopy for purity.

Dose: 300ug/mouse

Poly-L-Arginine (pR) Poly-L-Arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100 ug/mouse

Polyinosinic-polycytidylic polyinosinic-polycytidylic acid acid (pIC) (Sigma Chemicals, P-0913, Lot 125H4024) with the molecular weight ranging from 220,000 to 460,000

Dose: 100 ug/mouse

Experimental groups (4 mice per group) 1. P1A-peptide + pIC + pR 2. P1A-peptide + pIC 3. P1A-peptide + pR

On day 0 mice were injected into each hind footpad with a total volume of 100 ul (50 ul per footpad) containing the above mentioned compounds. Animals were sacrificed 4 days after injection and popliteal

lymph nodes were harvested. Preparation of lymph nodes and IFN- γ ELISPOTs were carried out as described in example 1. Results are expressed as the number of spots/1x10⁶ cells SD of triplicates. As shown in Figure 5, the combined application of pIC and pR induces strong antigen-specific response also in another mouse strain.

Example 6

The combined application of oligo-dIC26 and pR synergistically enhances the immune response against ovalbumin-derived peptide.

Mice C57B1/6 (Harlan/Olac)

Peptide OVA257-264-Peptide (SIINFEKL), an MHC class I(H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), was synthesized using standard solid phase F-moc synthesis,

HPLC purified and analyzed by mass spectroscopy for purity.

Dose: 300 μ g/mouse

Poly-L-Arginine (pR) Poly-L-Arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100 μ g/mouse

Oligo-deoxy IC, 26-mer oligo-dIC was synthesized by standard (oligo-dIC26) standard phosphoamidite chemistry on a

4 μ mol scale and purified by HPLC (NAPS Göttingen, Germany)

Dose: 5 nmol/mouse

Experimental groups (4 mice per group) 1. OVA-peptide-f-oligo-dIC + pR 2. peptide + oligo-dIC26 3.

OVA257-264-peptide + pR

On day 0 mice were injected into each hind footpad with a total volume of 100 μ l (50 μ l per footpad) containing the above mentioned compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Preparation of lymph nodes and IFN- γ ELISPOTs were carried out as described in example 1. Results are expressed as the number of spots/1x10⁶ cells SD of triplicates. As shown in Figure 6, the combined application of oligo-dIC26 and pR induces strong peptide-specific T cell response.

Example 7

The antigen-specific immune response against ovalbumin-derived peptide induced by combined injection of poly-L-arginine (pR) and polyinosinic-polycytidylic acid (pIC) is systemic.

Mice C57B1/6 (Harlan/Olac)

Peptide OVA257-264-peptide (SIINFEKL), an MHC class I(H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), was synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spectroscopy for purity.

Dose: 300 μ g/mouse

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals,

P-4663, Lot 68H5903; with the average molecular weight (MW) 10,000

Dose: 100 μ g/mouse

Polyinosinic-polycytidylic acid (pIC) (Sigma Chemicals,

P-0913, Lot 109H4037) with the molecular weight between 220,000 to

460,000 (average length: 500 bp)

Dose: 100 μ g/mouse

Experimental groups (8 mice per group) 1. OVA257-264-peptide + pIC + pR 2. OVA257-264-peptide +

pIC 3.OVA257-264-peptide + pR

On day 0, mice were injected either into hind footpads or into the flank with a total volume of 100 μ l (50 μ l per each footpad, 100 μ l per flank) containing the above listed compounds. Four mice per group were sacrificed 4 days after injection and draining lymph nodes (popliteal and inguinal lymph nodes for footpad and flank injection, respectively) were harvested. Preparation of lymph nodes and IFN- γ ELISPOTs were carried out as described in example 1.

As already shown in previous examples, by injecting OVA-derived peptide in combination with pIC and pR, strong peptide-specific T cell response could be induced on day 4 in draining lymph nodes. To investigate whether the immune response induced by one single injection of peptide with pIC/pR is systemic, the rest of the animals (four per group) was bled from the tail vein at selected time points after injection, peripheral blood leukocytes (PBLs) were isolated, re-stimulated either with the relevant peptide, pIC, pR, ConA (positive control) or medium alone (background) and the number of IFN- γ -secreting T lymphocytes was determined using an ELISPOT assay. Assays were carried out in duplicates. Each spot represents a single IFN- γ -producing

T cell. Spots were counted using a Biosys reader (Biosys, Germany). Number of background spots was subtracted from all samples. Results are expressed as the number of spots/ 1×10^5 cells + SD of duplicates. After the stimulation of PBLs with ConA, many spots demonstrating a good condition of the used lymphocytes could be detected. These results showed that injection of peptide with pIC/pR indeed results in the systemic response as observed on day 7 in peripheral blood (Figure 7A, B). In contrast, there was almost no or very weak peptide-specific immune response detectable on day 7 when mice were injected with peptide and pR or peptide and pIC. The strong systemic response induced by single injection of peptide with combination of pR/pIC declined rapidly within the next 30 days.

To determine whether any component of the vaccine could have undesired effects for the host, e. g., induce the systemic release of pro-inflammatory cytokines, animals were injected into hind footpads with combinations as mentioned before, sera from mice were collected one hour after injection and were screened for TNF- α and IL-6 by ELISA. Neither TNF- α nor IL-6 could be detected in the serum of any of the mice, whether injected with peptide/pR, peptide/pIC or peptide and the combination of both substances.

These results indicate that the response induced by injection of peptide antigen with a mixture composed of pIC and pR is systemic.

Example 8

The combined injection of polyinosinic-polycytidylic acid (pIC) and various polycationic compounds synergistically enhances the immune response against ovalbumin-derived peptide.

Mice C57B1/6 (Harlan/Olac)

Peptide OVA257-264-peptide (SIINFEKL), an MHC class I (H-2K b)-restricted epitope of chicken ovalbumin (Rotzschke et al.,

1991), was synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spectroscopy for purity.

Dose: 300 μ g/mouse

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals, P

4663, Lot 68H5903; with the average molecular weight (MW) 10,000

Dose: 100 μ g/mouse

Poly-L-lysine (pL) Poly-L-lysine, SIGMA Chemicals, P

6516, Lot 78H5910; with the average

MM 9,500

Dose: 100ug/mouse Poly-L-ornithine (pO) Poly-L-ornithine, SIGMA Chemicals, P-4538, Lot 57H5515; with the average MW 10,000

Dose: 100pg/mouse

Diethylaminoethyl-dextran DEAE-dextran, chloride form, pr(DEAE-dextran) pared from dextran of the average molecular weight 500,000; SIGMA Chemicals, D-9885, Lot39H1323

Dose: 100ug/mouse Polyinosinic-polycytidylic Polyinosinic-polycytidylic acid (Amacid(pIC) ersham Pharmacia Biotech, 27-4732, Lot6034732012)

Dose: 50ug/mouse

Experimental groups (4 mice per group) 1.OVA257-264-peptide + pIC + pR 2.OVA257-264-peptide + pIC 3.OVA2s7-264-peptide + pR 4.OVA257-264-peptide 5.OVA257-264-peptide + pIC + pL 6.VA2s7-264-peptide + pL 7.OVA257-264-peptide + pIC + pO 8.OVA257-264-peptide + pO 9.OVA257-264-peptide + pIC + DEAE-dextran 10.OVA257-264-peptide + DEAE-dextran

On day 0, mice were injected into each hind footpad with a total volume of 100 ul (50pi per footpad) containing the above listed compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph node cell suspensions were prepared and IFN- γ ELISPOTs were performed as described in

Example 1. Results are expressed as the number of spots/1x10⁶ cells SD of triplicates. As shown in Figure 8, injection of peptide in combination with pIC and any of the above listed polycationic compounds (pR, pL, pO, DEAE-dextran) leads to the strong peptide-specific response. In contrast, injection of peptide with either of the substances alone did not induce peptide-specific T cells. Interestingly, a 50-fold higher amount of DEAE-dextran has to be used in combination with pIC to induce the same numbers of peptide-specific IFN- γ -producing T cells as injection of pIC with any of other used polycationic compounds (pR, pL, pO).

Example 9

The combined injection of ovalbumin (OVA) with poly-L-arginine (pR) and polyinosinic-polycytidylic acid (pIC) synergistically enhances the OVA-specific immune response.

Mice C57B1/6 (Harlan/Olac)

Ovalbumin (OVA) Ovalbumin from chicken egg, grade V, SIGMA Chemicals, A-5503, Lot 54H7070

Dose: 50 ug/mouse

Peptides OVA257-264-peptide (SIINFEKL), an MHC class I (H-2Kb)-restricted dominant epitope of chicken ovalbumin (Rotzschke et al., 1991), OVA265-280-peptide (TEWTSSNVMEERKIKV), an MHC class II (H-2Ab)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991) were synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spec troscopy for purity. Dose used for the re-stimulation of lymph node cells: 10Vg/ml.

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals, P

4663, Lot 68H5903

Dose: 100ug/mouse

Polyinosinic-polycytidylic Polyinosinic-polycytidylic acid (Amacid (pIC) ersham Pharmacia Biotech, 27-4732,

Lot 6034732012)

Dose: 50ug/mouse

Experimental groups (4 mice per group) 1. OVA + pIC + pR 2. OVA + pIC 3. OVA + pR 4. OVA

On day 0, mice were injected into each hind footpad with a total volume of 100 pi (50 pi per footpad)

containing the above listed compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph node cell suspensions were prepared and IFN- γ ELISPOTs were performed as described in

Example1. Results are expressed as the number of spots/1x10⁶ cells SD of triplicates. As shown in Figure 9, injection of ovalbumin (OVA) with either pIC or pR leads to the antigen-specific immune response when compared with injection of OVA alone.

However, when OVA is injected with a mixture of pIC and pR, the synergizing effect of both substances can be observed, resulting in enhanced antigen-specific T cell response. Importantly, the IFN- γ production was detected not only upon the re-stimulation with the whole OVA protein but also with both, MHC class I(OVA257-264)-and II(OVA2ss-280)-restricted OVA-derived epitopes (Figure 9). These data demonstrate that using the combination of pIC and pR, not only peptides but also whole proteins can be used as an antigen for the vaccine composition.

Example 10

Poly-L-arginine does not affect polyinosinic-polycytidylic acid(pIC)-induced in vitro maturation of DCs.

Lipopolysaccharide (LPS) Lipopolysaccharide from Escherichia coli; serotype 055: B5 (SIGMA Chemicals)

Dose: 1ug/ml

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals, P-4663, Lot 68H5903

Dose: 10ug/ml

Polyinosinic-polycytidylic acid Polyinosinic-polycytidylic acid (pIC) (Amersham Pharmacia Biotech, 27 4732, Lot 6034732012)

Dose: 10 pg/ml

Experimental groups 1. medium 2., LPS 3. pR 4. pIC 5. pR+pIC

It has previously been described that pIC, similar to influenza virus infection, triggers maturation of human DCs in vitro (Cella et al, 1999; Verdijk et al., 1999). Human monocyte-derived DCs were used to investigate how poly-L-arginine affects this pIC-induced DC maturation. Human DCs were generated from monocytes.

Briefly, peripheral blood leukocytes (PBLs) were isolated from buffy coats of healthy volunteers by Ficoll gradient centrifugation. Monocytes were isolated from PBLs using CD14-coated magnetic beads (Miltenyi Biotec Inc., Germany) applied according to the manufacturer's instructions. Using this method, we obtained > 95% CD14+ cells as determined by flow cytometry. These CD14+ monocytes were cultured in RPMI 1640 medium supplemented with 10% FCS (PAA Laboratories, Linz, Austria), non-essential aminoacids, L-glutamin, gentamycin, sodium pyruvate, 100 ng/ml human GM-CSF and 500 U/ml human IL-4 in 6-well tissue plates for 6-7 days. To this end, the cultures contained > 80% MHC class II+/CD1a+ cells (=DCs).

To determine phenotypic maturation, day 6-cultured DCs were stimulated either with pIC, pR or with a combination of both substances for 48 hours and were analyzed for the expression of several surface molecules by flow cytometry. For control purposes,

DCs were also stimulated with LPS or were left untreated. As shown in Figure 10, pIC induced an up-regulation of HLA-DR, -DQ and HLA-I molecules, co-stimulatory molecules such as CD40, CD54, CD80, de-novo expression of CD86 and the maturation marker CD83 as well as a down-regulation of CD1a molecules when compared to untreated DCs. The maturation effect of pIC was in all cases comparable to that induced by LPS. In addition, this analysis revealed a slight up-regulation of CD11a, CD11c, CD13, CD25, CD29 and CD50 antigens on DCs upon pIC stimulation. None of the above described phenotypic changes could be observed when DCs were incubated with pR alone. The phenotype of DCs

stimulated with a mixture of pIC and pR was similar to that induced by pIC alone (Figure 10).

Therefore, pIC has the capacity to induce maturation of human DCs in vitro, and pR does not affect this pIC-induced differentiation process.

Example 11

The antigen-specific immune response induced by the combined application of antigen with oligo-deoxyIC26-r and poly-L-a, ginine (pR) is systemic.

Mice'C57B1/6 (Harlan/Olac)

PeptideOVA257-264-peptide (SIINFEKL), an MHC class I(H-2Kb)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991), was synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spectroscopy for purity.

Dose:300 ug/mouse

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals

Dose: 100 ug/mouse

Oligo-deoxy IC, 26-meroligo-dIC26-mer was synthesized by (oligo-dIC26-mer) standard phosphoamidid chemistry on a 11 mol scale and purified by HPLC (NAPS Göttingen, Germany)

Dose: 5nmol/mouse

Experimental groups (8 mice per group) 1.OVA257-264-peptide + oligo-dIC26-mer + pR 2.

OVA257-264-peptide + oligo-dIC26-mer 3.OVA257-264-peptide + pR4. tide

On day 0, mice were injected into each hind footpad with a total volume of 100 ul(50 ul per footpad) containing the above listed compounds. Animals (4 mice/group) were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph node cell suspensions were prepared and IFN- γ ELISPOTs were performed as described in Example1.

As already shown previously, the combined application of peptide with oligo-dIC26-mer and pR induced strong antigen-specific response against OVA-derived peptide on day 4 in draining lymph node cells. To examine whether the immune response induced by one single injection of peptide with oligo-dIC/pR is systemic, the rest of the mice (4 mice/group) was investigated at selected time points after injection for the presence of peptide-specific IFN- γ -producing T cells in spleen or peripheral blood using an IFN- γ ELISPOT assay. Results are expressed as the number of spots/1x10⁶ cells SD of duplicates. Figure 11 shows that the response induced by the injection of OVA-derived peptide with oligo-dIC/pR is systemic and lasts at least until day 59 after one single injection (the latest time point of investigation). In contrast, the local or systemic response could not be observed when peptide was injected alone, with oligo-dIC26-mer or pR.

To determine whether any component of the vaccine could have undesired effects for the host, e. g., induce the systemic release of pro-inflammatory cytokines, animals were injected into hind footpads with combinations as mentioned before, sera from mice were collected one hour after injection and were screened for

TNF- α and IL-6 by ELISA. Neither TNF- α nor IL-6 could be detected in the serum of any of the mice, whether injected with peptide/pR, peptide/oligo-dIC or peptide and the combination of both substances.

These results indicate that the response induced by injection of peptide antigen with a mixture composed of oligo-dIC and pR is systemic.

Example 12

The combined application of oligo-deoxyIC26mOr and poly-L-arginine (pR) enhances the ovalbumin (OVA)-specific T cell response.

MiceC57B1/6 (Harlan/Olac)

Ovalbumin (OVA) Ovalbumin from chicken egg, grade V,
SIGMA Chemicals, A-5503, Lot 54H7070

Dose: 50ug/mouse

Peptides OVA25 7-264-peptide (SIINFEKL), an MHC class I(H-2Kb)-restricted dominant epitope of chicken ovalbumin (Rotzschke et al., 1991), OVA265-2so-peptide (TEWTSSNVMEERKIKV), an MHC class II (H-2Ab)-restricted epitope of chicken ovalbumin (Rotzschke et al., 1991) were synthesized using standard solid phase F-moc synthesis, HPLC purified and analyzed by mass spec troscopy for purity. Dose used for the re-stimulation of cells:

10ug/ml.

Poly-L-arginine (pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues; SIGMA Chemicals,

P-4663, Lot 68H5903

Dose: 100Vg/mouse

Oligo-deoxy IC, 26-mer oligo-dIC26-mer was synthesized by (oligo-dIC26-mer) standard phosphoamidite chemistry on a 4umol scale and purified by HPLC (NAPS Göttingen, Germany)

Dose: 5 nmol/mouse

Experimental groups (8 mice per group) 1. OVA + oligo-dIC26-mer + pR 2. OVA + oligo-dIC26-mer 3. OVA + pR 4. OVA

On day 0, mice were injected into each hind footpad with a total volume of 100pi (50 ul per footpad) containing the above listed compounds. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph node cell suspensions were prepared and IFN- γ ELISPOTs were performed as described in Example 1. Results are expressed as the number of spots/1x10⁶ cells SD of triplicates. As shown in Figure 12A, injection of ovalbumin (OVA) with a mixture of oligo-dIC and pR results in enhanced antigen-specific T cell response.

Importantly, the IFN- γ production was detected not only upon the re-stimulation with the whole OVA protein but also with both,

MHC class I(OVA257-264)-and II(OVA265-280)-restricted OVA-derived epitopes (Figure 12A).

To further examine whether the immune response induced by one single injection of OVA protein with oligo-dIC/pR is systemic, the rest of the mice (4 mice/group) was investigated at selected time points after injection for the presence of peptide-specific IFN- γ -producing T cells in peripheral blood using an IFN- γ ELISpot assay. Results are expressed as the number of spots/1x10⁵ cells SD of duplicates. Figure 12B shows that the response induced by the injection of OVA with oligo-dIC/pR is systemic and persisting at least until day 97 after injection (the latest time point of investigation). In contrast, the local or systemic response could not be observed when OVA was injected alone, with oligo-dIC26-mer or pR.

These data demonstrate that using the combination of oligo-dIC and pR, not only peptides but also whole proteins can be used as an antigen for the vaccine composition and that the response induced by injection of protein antigen with a mixture composed of oligo-dIC and pR is systemic and longer-lasting.

Example 13

The combined application of oligo-deoxyICze-mar and poly-L-arginine (pR) enhances the ovalbumin (OVA)-specific humoral response.

MiceC57B1/6 (Harlan/Olac)

Ovalbumin (OVA) Ovalbumin from chicken egg, grade V,
SIGMA Chemicals, A-5503, Lot 54H7070

Dose: 50 ug/mouse

Poly-L-arginine(pR) Poly-L-arginine with an average degree of polymerization of 60 arginine residues;
SIGMA Chemicals,
P-4663, Lot 68H5903

Dose: 100ug/mouse

Oligo-deoxy IC, 26-meroligo-dIC26-mer was synthesized by(oligo-dIC26-mer) standard phosphoamidite chemistry on a 4 pmol scale and purified by HPLC (NAPS Göttingen, Germany)

Dose: 5nmol/mouse

Experimental groups (4 mice per group) 1. OVA +oligo-dIC26-mer + pR 2. OVA +oligo-dIC26-mer 3. OVA + pR 4. OVA

On day 0, mice were injected into each hind footpad with a total volume of 100 μ l (50 μ l per footpad) containing the above listed compounds. On day 24 after injection, serum was collected and screened by ELISA for the presence of OVA-specific antibodies.

These results show that the injection of OVA in combination with oligo-dIC and pR enhanced the production of OVA-specific IgG antibodies when compared with injection of OVA with each of the substances alone (Figure 13A, B). Interestingly, titers of both

IgG2a and IgG1 were increased upon one single injection of OVA with oligo-dIC/pR, implying that both Th1 and Th2 cells were involved. However, after 115 days only the increased IgG2a levels were still detectable in sera of mice injected with OVA and oligo-dIC/pR.

These data demonstrate that the combined injection of OVA with oligo-dIC and pR enhances the OVA-specific humoral response.

This response is characterized by the production of both Th1-and Th2-induced antibody isotypes in the early phase, but later, mainly by Th1-induced antibodies.

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Claims of WO0193903

 Translate this text

C 1 a i m s : 1. Pharmaceutical composition comprising a T cell epitope or a mixture of T cell epitopes a polycationic peptide and a nucleic acid based on inosine and cytosine.

2. Composition according to claim 1, characterized in that the nucleic acid based on inosine and cytosine is selected from poly I: poly C, poly IC, poly dC: polydI and poly dIC.
3. Composition according to claim 1 or claim 2, characterized in that the polycationic peptide is selected from polyarginine and polylysine or a polypeptide containing at least 50% basic amino acid residues, especially arginine or lysine residues.
4. Composition according to anyone of claims 1 to 3, characterized in that the polycationic peptide contains more than 5 residues, preferably between 10 and 1000, especially between 50 and 500.
5. Composition according to anyone of claims 1 to 4, characterized in that the T cell epitope is derived from a human, animal or plant pathogen.
6. Composition according to anyone of claims 1 to 4, characterized in that the T cell epitope is derived from a viral or bacterial pathogen.
7. Composition according to anyone of claims 1 to 6, characterized in that the antigen is a protein, carbohydrate or lipid antigen or a mixture thereof.
8. Composition according to anyone of claims 1 to 7, characterized in that the T cell epitope is derived from parasites or plant pathogens.
9. Composition according to anyone of claims 1 to 8, characterized in that the T cell epitope is a peptide consisting of 6 to 20, preferably 7 to 15, especially 8 to 11, amino acid residues.
10. Composition according to anyone of claims 1 to 9, characterized in that it contains 1 ng-1 g, especially 1-10000ug, T cell epitope (peptide)
1 ng-1 g, especially 0.1-1000ug, polycationic peptide and
1 ng-1 g, especially 10ug-300 mg, nucleic acid based on inosine and cytosine.
11. Composition according to anyone of claims 1 to 10, characterized in that it contains additional substances selected from auxiliary substances and further effective substances.
12. Use of a composition according to anyone of claims 1 to 11 for the preparation of vaccine.
13. Use of a composition according to any one of claims 1 to 11 for the preparation of a vaccine inducing a systemic immune response.
14. Kit for vaccination comprising a component containing a T cell epitope or a mixture of T cell epitopes and a polycationic peptide and a component containing a nucleic acid based on inosine and cytosine.

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